REMARKS

Claims 1-5, 7, 14-16, 18-24, 26-29, 31, 32, 40, 46, 47, 52, 54 and 56 are pending in the present application. Claims 3, 14, 22-24, 27, 46, 47, 52, 54 and 56 were withdrawn from consideration. Applicants believe that claim 14 was inadvertently marked as withdrawn in the Office Action. In the Restriction Requirement mailed June 1, 2007, claim 14 was included in Group I, the elected group, and was not subject to further restriction. Applicants respectfully submit that claim 14 should not have been withdrawn. By virtue of this response, claim 7 has been cancelled and claims 1, 14, 15, 16, 18, 19, 20, 23, 26, 28 and 40 have been amended. Accordingly, claims 1-5, 14-16, 18-24, 26-29, 31, 32, 40, 46, 47, 52, 54 and 56 are currently under consideration. Amendment and cancellation of certain claims is not to be construed as a dedication to the public of any of the subject matter of the claims as previously presented. Support for amendments of claims 1, 14 and 15 is found throughout the specification, at least at paragraphs [0011], [0178], [0182], [0184] – [0188] of the published application, US 2006/0233771 A1.

Claim Rejections – 35 U.S.C. § 112, First Paragraph

Claims 1, 2, 4, 5, 7, 16, 16, 18-21, 26, 28, 29, 31, 32, and 40 are rejected under 35 U.S.C. 112, first paragraph. The Examiner alleges that the specification, while being enabling for an in vitro method of differentiating a rat E14.5 cell from the ventral mesencephala into a dopaminergic neuron, wherein the method comprises: treating the cell with Wnt5a, wherein the cell differentiates into a TH expressing cell, does not reasonably provide enablement for an in vitro method of differentiating any stem, neural stem, progenitor, precursor, or neural cell from any species of animal, other than rat, wherein the method comprises: introducing a transgene construct comprising a nucleic acid sequence encoding Nurr1 into the cell, and treating the cell with any Wnt ligand, wherein the cell differentiates into a dopaminergic neuron.

Applicants respectfully disagree with the Examiner's contention that the claims are not fully enabled for its scope. To comply fully with the enablement requirements of § 112, first paragraph, a specification must adequately teach how to make and use the claimed invention without undue experimentation. MPEP §2164.01(a) states that "[t]here are many factors to be considered when

determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is "undue." These factors include: (a) the breadth of the claims; (b) the nature of the invention; (c) the state of the prior art; (d) the level of one of ordinary skill; (e) the level of predictability in the art; (f) the amount of direction provided by the inventor; (g) the existence of working examples; and (h) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. A few of the Wands factors are discussed in greater detail below. However, the absence of discussion of the other Wands factors does not mean that Applicants believe that those factors do not also weigh in the Applicant's favor.

Breadth of Claims

Without acquiescing to this § 112, first paragraph, enablement rejection, and solely in an effort to expedite prosecution, Applicants have amended claim 1 to methods of inducing or promoting dopaminergic neuronal development by enhancing proliferation, self-renewal, dopaminergic induction, survival, differentiation and/or maturation in a neural stem, progenitor or precursor cell, or other stem cell, comprising expressing a nuclear receptor of the Nurr1 subfamily above basal levels within the cell, and treating the cell with a Wnt-5a ligand, thereby producing or enhancing proliferation, self-renewal, survival and/or dopaminergic induction, differentiation, survival or acquisition of a neuronal dopaminergic phenotype. With respect of breadth of claims, support for this breadth of the claim is found throughout the specification. For example, enhancing induction of a dopaminergic phenotype in neural stem cells, neural precursors or neural progenitor cells by treatment with Wnt-5a is provided, at least, at paragraphs [0011], [0178], [0182], [0184] – [0188]. Support for enhancing proliferation and self-renewal in neural stem cells, neural precursors or neural progenitor cells by treatment with Wnt-5a is provided, at least, at paragraphs [0178], [0186] and [0188]. Support for induction of a dopaminergic phenotype in neural stem cells, neural precursors or neural progenitor cells that overexpress Nurr1 by treatment with Wnt-5a is provided, at least, at paragraph [0184] and discussed below. As such, the claims are within the scope of the specification.

Level of Predictability of the Art

The Examiner alleges that at the time of the filing, the art taught that differentiation of an undifferentiated cell into a specific cell type was unpredictable. The Examiner compares the results of Sakurada *et al.* (1999) *Development* 126:4017-4026, with those of Wagner *et al.* (1999) *Nat. Biotechnol.* 17:653-659. In the case of Sakurada *et al.*, Nurr1 was overexpressed in neural progenitor cells from adult rat hippocampus resulting in expression of tyrosine hydroxylase (TH) but not in the induction of dopaminergic neurons. Wagner *et al.* discloses overexpression of Nurr1 in neural progenitor cells from mouse cerebellum but TH expression was not seen until additional factors were provided by co-culture with rat ventral mesencephalon. The Examiner relies on the hypothesis of Chung *et al.* (2002) *Eur. J. Neurosci.* 16:1829-1838 that expression of Nurr1 leads to TH expression by "seemingly different mechanisms." The Examiner alleges that this difference between the teachings of Sakurada, *et al.* and Wagner, *et al.* indicate the art was unpredictable at the time of filing. Applicants respectfully disagree. The present invention is based, in part, on the discovery that Nurr1 expression is an early step in dopaminergic neuron differentiation. Neither Sakurada *et al.* nor Wagner *et al.* disclose that Nurr1, by itself, is sufficient to differentiate neural progenitor cells. This is corroborated by Park *et al.* (2006) *FASEB J.* 20:2553-2555 which discloses on page E1910 "Nurr1 is not sufficient to induce fully mature DA neuron progeny from neural precursors and that additional factors are required to yield DA neurons capable of restoring in vivo function." A number of studies report the differentiation Nurr1-derived dopaminergic neurons from a number of different sources including the rat ventral mesencephalon (present specification), mouse ventral mesencephalon (Parish *et al*, (2008) *J. Clin. Invest.* 118:149-159), mouse cerebral neural progenitor cells (present specification, WO 00/66713, and Wagner *et al.*), murine ES cells (Chung *et al.*; Kim et al. (2002) Nature 418: 50-56; Martinet, et al. (2006) *Proc. Natl. Acad. Sci. USA* 103:2874-2882), rat cortical cells (Park *et al.*), rat adult subventricular zone cells (Shim *et al.* 2007 *Stem Cells* 25:1252-1262), and human ES cells (Martinet, *et al.* 2006). Taken together, the role of Nurr1 as an early step in dopaminergic neural differentiation from a number of different progenitor cells and from a number of species rather than the expression of TH in undifferentiated

neural progenitor cells is indicative of the predictability of art related to dopaminergic neural differentiation. As such, the claims are within the predictability of the art.

Amount of Direction

The Examiner alleges that the specification nor the art provide guidance that introducing a Nurr1 expression construct and treatment with Wnt-5a are sufficient to induce a stem cell to develop into a dopaminergic neuron. Applicants respectfully disagree. As described in paragraph [0168], the experimental results set out in WO 00/66713 are specifically incorporated by reference. These experimental results demonstrate proliferation and/or self-renewal of dopaminergic precursors and induction of dopaminergic neurons in stem, neural stem, precursor or progenitor cells expressing Nurr1, in the presence of type 1 astrocytes or glial cells. In particular, WO 00/66713, disclosed overexpression of Nurr1 in neural stem cells isolated from developing mouse cerebellum. Paragraph [0076] of the present specification provides guidance regarding a pretreatment step of expressing Nurr1 above basal levels. As disclosed in paragraph [0184], Wnt-5a treatment induced a significant increase in the proportion of Nurr1 positive cells that express TH. Furthermore, as disclosed in paragraph [0185], treatment of Nurr1 positive cells with Wnt-5 induced expression of Ptx3 mRNA, a dopaminergic marker. Taken together, the specification provides guidance to enhance the induction of dopaminergic neurons from Nurr1 positive stem cells.

The Examiner asserts that, while the specification teaches that supernatants containing Wnt-5a can induce TH expression, the teaching does not indicate that only Wnt-5a is required for TH expression. Applicants point out that the invention, as claimed, relates to methods of inducing dopaminergic neurons by enhancing differentiation in a neural stem, progenitor, precursor cell, or other stem or neural cell comprising expressing a nuclear receptor of the Nurr1 subfamily above basal levels within the cell and treating the cells with Wnt-5a ligand thereby enhancing dopaminergic induction, differentiation or acquisition of a dopaminergic neuronal phenotype. Partially purified supernatants, enhanced for Wnt5a content, were compared to similar supernatants lacking Wnt-5a, indicating a specific effect of Wnt-5a on the enhanced induction of TH expression in Nurr1 positive stem cells (see paragraph [0184]). As such, the specification provides ample

guidance to the enhanced differentiation of Nurr1 positive neural stem cells by treatment with Wnt-5a.

The Examiner contends that given the teachings of Sakuruda *et al.* and Wagner *et al.* an artisan cannot readily extrapolate which cell types should be treated with other factors such as activator of RXR, bFGF/FGF8/Shh, antioxidants or early glial cells without further guidance. The specification provides ample guidance for testing what other factors may enhance differentiation of different cell types. Paragraphs [0073] to [0080] provide guidance to test and evaluate what other factors may be used to enhance differentiation into dopaminergic neurons. As such, it would not require undue experimentation to test the factors recited in claims 16, 18, 19 and 20, in any cell modified to express Nurr1 above basal levels and treated with Wnt-5a ligand.

The Examiner contends that the teachings of Sakurada *et al.* and Wagner *et al.* do not indicate that the TH expressing cells can be transplanted into a host and survive. Applicants point to page 658 of Wagner *et al.*, second paragraph on the left, which states "a few c42-derived dopaminergic cells displayed a high level of differentiation and apparent integration into the host tissue" showing that after dopaminergic induction, their phenotype is stable. In addition, Applicants would like to direct the Examiner's attention to Parish *et al.* (2008) *J. Clin. Invest.* 118:149-159. Here, dopaminergic cells induced from murine ventral mesencephalon cells by treatment with Wnt-5a were transplanted into brains of parkinsonian mice. A significant portion of implanted cells survived for at least eight weeks and imparted functional correction of the parkinsonian defects in the mice. In addition, direction towards transplanting dopaminergic neurons prepared by the methods of the invention is provided in paragraphs [0190] to [0192] of the specification.

On page 13 of the Office Action, the Examiner alleges that the specification does not provide guidance on how to transdifferentiate any neuron to a dopaminergic cell. Applicants respectfully disagree but without acquiescing to this § 112, first paragraph, enablement rejection, and solely in an effort to expedite prosecution, Applicants have amended claims 1, 14, 15, 16, 20, 23 and 26 to recite neural stem, progenitor or precursor cell, or other stem cells.

The Examiner alleges that the specification does not provide significant guidance regarding obtaining cells from any species of animal and differentiate them into specific cell types under specific culture conditions. The Examiner cites Ostenfeld *et al.* (2002) *Dev. Brain Res.* 134:43-55 as an example of differences in cell growth and differentiation of neural progenitor cells between rodents and humans. Very few TH positive cells; however, were derived from either rodent or human neurospheres. The Examiner also cites Pera *et al.* (2000) *J. Cell Sci.* 113:5-10 and points out that whereas rodent stem cells can be maintained in the presence of LIF, human embryonic stem cells do not respond to LIF. This difference, however, is related to the maintenance of an undifferentiated state rather than differentiation into a dopaminergic phenotype. As discussed above, differentiation of dopaminergic neurons has been demonstrated by overexpression of Nurr1 in rats, mice and humans; thus, using a pathway similar to the pathway described in the present specification. Applicants point to Perrier *et al.* (2004) *Proc. Natl. Acad. Sci. USA* 101:34:12543-12548 which summarized on page 12547 that "later studies in mouse and nonhuman primate ES cells demonstrated that the neural-inducing effects are separate from the effects on patterning and that differentiation conditions can be adapted for a wide range of neuronal and glial subtypes." In addition, convergence between Nurr1 transcriptional activation and Wnt signaling in rodent and human systems is provided by Kitagawa *et al.* (2007) *Mol. Cell. Biol.* 27:7486-7496. As such, Applicants provide guidance for the enhanced induction of dopaminergic neurons from neural stem, progenitor or precursor cell, or other stem cells expressing Nurr1 above basal levels and treating the cell with a Wnt-5a ligand from a number of species.

On page 15 of the Office Action, the Examiner alleges that the specification teaches that Wnt-5a was the only Wnt protein that differentiated cells from rat E14.5 ventral mesencephala. Applicants respectfully point out that claim 1, as originally presented, did not limit Wnt treatment to the induction of differentiation. However, without acquiescing to this § 112, first paragraph, enablement rejection, and solely in an effort to expedite prosecution, Applicants have amended claim 1 to methods of inducing or promoting dopaminergic neuronal development by enhancing proliferation, self-renewal, dopaminergic induction, survival, differentiation and/or maturation in a neural stem, progenitor or precursor cell, or other stem cell, comprising expressing a nuclear

receptor of the Nurr1 subfamily above basal levels within the cell, and treating the cell with a Wnt-5a ligand, thereby producing or enhancing proliferation, self-renewal, survival and/or dopaminergic induction, differentiation, survival or acquisition of a neuronal dopaminergic phenotype.

The Examiner asserts that the specification does not provide guidance to enable a method of treating an individual comprising administering a composition of dopaminergic cells made by the claimed method. Applicants respectfully disagree. As discussed above, Wagner *et al.*, describe c42-derived dopaminergic cells displayed apparent integration following implantation into the host tissue. Also as discussed above, Applicants direct the Examiner's attention to Parish *et al.* (2008). Here, dopaminergic cells induced from murine ventral mesencephalon cells by treatment with Wnt-5a were transplanted into brains of parkinsonian mice. A significant portion of implanted cells survived for at least eight weeks and imparted functional correction of the parkinsonian defects in the mice. Parish *et al.* also reference clinical trials with transplantation of human fetal mesencephalic tissue in Parkinson's patients demonstrated that grafted dopaminergic neurons can reinnervate the denervated striatum, release dopamine, and become functionally integrated into host neural circuitries (see Introduction). These references indicate that at the time of filing of the present application, methods of implanting cells in the brains of human patients were known in the art. In addition, direction towards transplanting dopaminergic neurons prepared by the methods of the invention is provided in paragraphs [0190] to [0192]. As such, the specification and art provide guidance to treat individuals with cells made by the methods of the invention.

The Examiner alleges that except for Parkinson's disease, nothing in the specification teaches a relationship between dopaminergic neurons and other neurodegenerative diseases (e.g. Alzheimer's disease). Without acquiescing to this § 112, first paragraph, enablement rejection, and solely in an effort to expedite prosecution, Applicants have amended claim 40 to recite neurodegenerative diseases related to dopaminergic neuronal loss.

Existence of Working Examples

Applicants disclose working examples of the enhancement of induction of a dopaminergic neural phenotype by treatment of neural progenitor cells with Wnt-5a (See, for example, paragraphs [0173] to [0189]. These examples show that partially purified Wnt-5a enhanced induction of a dopaminergic neural phenotype from neural progenitor cells from rat ventral mesencephala.

In balance, Applicants believe that, in view of an analysis of the Wands factors, the claims are fully enabled commensurate in scope of the claims. In view of the foregoing, Appllicants respectfully request that the Examiner withdraw this rejection.

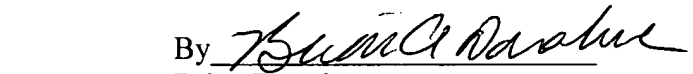
CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 441472001300. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: May 19, 2008

Respectfully submitted,

By 

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